

Discriminating tastes: self-selection of macronutrients in two populations of grasshoppers

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Abstract. The capacity to self-select an optimal balance of macronutrients (protein and carbohydrate) is studied in two populations of *Melanoplus sanguinipes* F. (Orthoptera: Acrididae). One population derives from the subarctic (interior of Alaska) and the other from the temperate zone (Idaho, U.S.A.). Over the duration of the fourth and fifth stadia, Alaskan grasshoppers consistently self-select a diet centred on a 0.90 ratio of protein : carbohydrate, whereas protein and carbohydrate intake by the Idaho grasshoppers is contingent on the particular food choices presented to them. When restricted to imbalanced diets, the Alaskan grasshoppers develop more rapidly than the Idaho grasshoppers, regardless of diet composition. The Idaho grasshoppers also have a greater amount of lipid than the Alaskan grasshoppers across all diets. Performance measures (body mass, survival, developmental times) are more sensitive to dietary imbalances in the Alaskan grasshoppers than in the Idaho grasshoppers. When fed diets with low, but balanced, proportions of protein and carbohydrate, grasshoppers of both populations are able to increase consumption to compensate for the low concentration of nutrients. The results suggest that demographic responses of insects to changes in host plant quality, such as may result from climate change, may differ among populations within a species.

Key words. Acrididae, Orthoptera, insect nutrition.

Introduction

Study of the nutritional ecology of insects has a long history (Waldbauer, 1968; Scriber & Slansky, 1981). More recently, researchers have gained an appreciation of the importance of the balance of macronutrients in an insect's diet, especially carbohydrates and protein (Raubenheimer & Simpson, 1993, 2003; Lee *et al.*, 2004; Simpson *et al.*, 2004). Allowed to self-select the composition of their diet, many insects consume carbohydrate and protein in proportions that are near optimal in terms of fitness for the organism (Waldbauer & Friedman, 1991; Simpson *et al.*, 2004). This self-selected balance of macronutrients is referred to as their 'intake target' (Raubenheimer & Simpson, 1993). Knowledge of an insect's intake target, and its survival, growth and fecundity

when unable to meet its intake target, would do much to increase our understanding of demographic responses to altered food quality resulting from seasonal variations or a changing climate.

Many investigations of insect nutrition have been prompted by concerns regarding the effects of elevated levels of atmospheric CO₂ on plant chemistry (Bezemer & Jones, 1998; Coviella & Trumble, 1999; Barbehenn *et al.*, 2004; Zvereva & Kozlov, 2006). Atmospheric concentration of CO₂ is expected to double within this century (Prentice *et al.*, 2001). Studies of C₃ plants exposed to elevated levels of CO₂ consistently show an increase in carbohydrates (up to 100%) and a decrease (up to 20%) in protein of leaf tissues (Korner & Miglietta, 1994; McGuire *et al.*, 1995; Poorter *et al.* 1997; Long *et al.*, 2004). These changes in leaf chemistry are often detrimental to insect herbivores (Bezemer & Jones, 1998; Coviella & Trumble, 1999; Asshoff & Hattenschwiler, 2005; Wu *et al.*, 2006), but insects can compensate for altered food quality in many ways: they may increase consumption in response to low levels of protein; they may increase the

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efficiency of protein assimilation or alter allocation of protein to different tissues; and they may use protein as an energy source if carbohydrates or lipids are limited (Zanotto *et al.*, 1993; Yang & Joern, 1994). Higher temperatures may also increase digestive efficiency of insects to override any effect of altered food quality (Zvereva & Kozlov, 2006).

Many species of locusts and grasshoppers (Orthoptera: Acrididae) are sensitive to food quality and populations may track food resources (Belovsky & Slade, 1995; Hunter *et al.*, 2001; Branson, 2003). The population dynamics of pest species of acridids may result from subtle changes in the collective performance of individuals. *Melanoplus sanguinipes* F. is perhaps the most economically damaging grasshopper in North America and has a very broad geographic distribution (Pfadt, 1995). Many field studies show *M. sanguinipes* to be a generalist feeder (Mulkern *et al.*, 1964; Pfadt *et al.*, 1988; Fielding & Brusven, 1992). Considering the economic importance of this species, there are relatively few studies of its nutritional requirements and demographic responses to diet quality using precisely defined diets. Joern & Behmer (1998) demonstrate the fecundity of adults of this species in response to different concentrations of nitrogen and carbohydrate in defined diets, but we are unaware of any such studies examining nymphal growth and development of *M. sanguinipes* in this context.

The broad geographic range of this species makes it a good candidate for comparative studies to examine adaptations to different environments (Fielding & DeFoliart, 2005). For the present study, one population of *M. sanguinipes* is derived from individuals collected from Idaho, U.S.A. (46.38°N, 117.02°W, 450 m elevation) and the other from the interior of Alaska (64.00°N, 145.73°W, 400 m elevation). The growing season at the Alaska source is typically approximately 80 days less than that of the Idaho location. Also, precipitation patterns differ between the locations, with the interior of Alaska receiving most of its annual precipitation in the summer, whereas, in Idaho, monthly mean precipitation is at a minimum during the summer months. Previous studies (Fielding & DeFoliart, 2007) show that nymphal development and growth occurs more rapidly in the Alaskan population under a variety of conditions. Furthermore, the Alaskan grasshoppers have a greater net retention of nitrogen than those from Idaho. In the present study, the geometrical framework of Raubenheimer & Simpson (1993, 1999, 2004) was employed to study the nutritional ecology of this species. The intake target for late-instar nymphs of these populations was unidentified, and performance on imbalanced foods was compared between these two populations adapted to very different climates.

Materials and methods

Second generation *M. sanguinipes*, derived from wild stock populations near Delta Junction, Alaska and Lewiston, Idaho were used in feeding trials. Hatchlings were reared in acetate tubes (9 cm in diameter \times approximately 50 cm in length). The ends of the tubes were capped with screened lids and one end of the tubes placed approximately 20 cm beneath

75-W incandescent lamps. Hatchlings were fed romaine lettuce and wheat bran through their first three stadia. Fourth-instar grasshoppers were weighed, sexed and placed into individual containers within 24 h of moulting.

The containers were plastic food-storage containers (12.5 \times 12.5 \times 5 cm). Five 3-mm diameter holes were drilled on each side for ventilation and the bottom was lined with 3.2-mm mesh hardware cloth, which was pressed into the bottom of the container leaving room so that frass could accumulate on the bottom of the container but grasshoppers would not come in contact with the frass. Dry meridic (a combination of natural and chemically defined ingredients) diet was provided in ceramic boats (57 \times 22 \times 11 mm). Free water was available to the grasshoppers at all times.

During the feeding trials, grasshoppers were housed in a Conviron growth chamber (Conviron TC16; Controlled Environments Limited, Winnipeg, Canada). The temperature followed a roughly sine-shaped curve from a maximum of 36 °C to a minimum of 18 °C. Temperatures in the chamber were at or above 30 °C for 13 h per day. Diurnally fluctuating temperatures were used for several reasons: to approximate the normal daily cycle experienced by grasshoppers in the field; to provide a range of temperatures in case populations differed in their optimum temperatures for growth and feeding; and to provide greater resolution in developmental times (grasshoppers were inspected daily after the temperature had dropped below 22 °C and it was assumed that any new adults had moulted during the previous warm cycle). The photoperiod comprised LD 16.5:7.5 h, with the dark hours centred around the minimum temperature.

Experimental diets were introduced to third-instar grasshoppers to familiarize them with it, but lettuce and bran were available throughout the third stadium. Starting on the first day of the fourth stadium, grasshoppers were fed solely on dry meridic diet (see below). The initial food given each grasshopper was weighed, as were any additional offerings. Grasshoppers were monitored daily so food and water could be replenished as needed. The date of the moult to adult was recorded and the grasshoppers were then weighed and frozen. Remaining food was collected and dried at 60 °C and weighed. Adults were also dried at 60 °C and dry weights obtained.

The experimental diets were all variations on the same base diet (Fielding & DeFoliart, 2007), which contained dried romaine lettuce, wheat germ, vitamins, yeast extract, cystine, glycine, cholesterol, flaxseed oil and cellulose. The base diet was supplemented with pure protein (casein) and/or pure carbohydrate (sucrose) and cellulose to achieve the various proportions and total concentrations of carbohydrate and protein. The diets were made by mixing together appropriate amounts of base diet, casein, sugar and cellulose and then dissolving them in distilled water to make a slurry to facilitate thorough mixing. The slurry was dried at 60 °C and ground with mortar and pestle.

In the experiment to determine self-selection of macronutrients, individuals were presented with a choice of two diets with differing proportions of protein and carbohydrate. Five different diets were formulated for the self-selection experiment consisting of different percentages of

protein and carbohydrate: (1) 60, 6; (2) 6, 48; (3) 12, 6; (4) 6, 24; and (5) 24, 6. Four pairs of food choices were tested: diets 1 and 2; 1 and 4; 2 and 3; 2 and 5. The two diets in each trial were offered in separate but identical containers side-by-side within the boxes. The choice experiments were conducted with six grasshoppers per treatment, and repeated twice, for a total of 18 grasshoppers per treatment.

Each population of grasshoppers was analyzed separately with multivariate analysis of variance (MANOVA) to evaluate the effects of diet choices (1 and 2, 1 and 4, etc.), sex, and trial on protein and carbohydrate consumption. Wilks' Lambda exact *F* statistic was used to assess overall significance of effects. Significant effects from the MANOVA were included in a univariate analyses of variance (ANOVA) for each dependent variable. Nonrandom selection of diets was tested for with *t*-tests of differences in the protein:carbohydrate ratio in the pair of diets combined versus that consumed by the grasshoppers. Variability in the intake of protein and carbohydrate over all diet choices was evaluated by comparing coefficients of variation between populations using the method of Miller (1991), as described in Zar (1999). The *Z*-test statistic is distributed as *t* with infinite degrees of freedom.

To examine grasshopper performance when restricted to unbalanced diets, grasshoppers were fed solely one of seven diets after they moulted to the fourth instar. These diets consisted of different percentages of protein and carbohydrate: (1) 10, 42; (2) 13, 36; (3) 16, 33; (4) 22, 26; (5) 27, 20; (6) 33, 16; and (7) 42, 10. To compare performance on a balanced, but low concentration of nutrients, two additional diets were included: 10 : 12% and 13 : 15% protein : carbohydrate. The no-choice experiments were conducted twice with five grasshoppers per treatment each time.

Lipid content was determined for each grasshopper to reach adult stage in the no-choice experiments. After drying, adult grasshoppers were ground with a mortar and pestle. The powder was weighed onto filter paper. The filter paper was folded and stapled shut and inserted into the thimble of a solvent extraction system (Soxtec 2043; Foss Analytical A/S, Eden Prairie, Minnesota). The thimbles were plugged with defatted cotton and submerged in boiling dichloromethane for 15 min, rinsed in the same solvent for 30 min, then the solvent allowed to drain for 10 min. Lipid was collected in pre-weighed stainless steel beakers, which were then dried and weighed again post-extraction. Samples from the Alaskan population on the 10 : 42 diet were inadvertently contaminated and were not included in the analyses involving lipids.

Protein and carbohydrate utilization were analyzed with ANCOVA, with nonlipid dry carcass weight or lipids (mg) as the dependent variable, population as a main factor, and protein or carbohydrate (mg) consumed as the covariable. Preliminary data indicated that the proportion of protein in the nonlipid mass of the grasshoppers did not differ among populations or with dietary intake. Visual inspection of the plotted data suggested that the relationship between protein consumption and nonlipid dry weight was nonlinear and so quadratic terms for protein consumption were included in the ANCOVA model.

Performance on unbalanced diets was assessed by ANOVA for each population of grasshoppers separately. Measures of performance when restricted to unbalanced foods included survival, duration of the fourth through fifth stadia, dry weight of adults and lipid-free dry weight of adults. To identify the performance plateau (the range of diets over which performance was not inhibited, Simpson & Raubenheimer, 1993), mean performance measures were compared with the best performance (shortest developmental times or greatest mass) using Dunnett's method (PROC GLM; SAS Institute, 2001). Performance on low protein, high carbohydrate diets (10 : 42 and 13 : 36) was compared with that on low protein balanced diets (10 : 12 and 13 : 16) with pairwise comparisons using Bonferroni corrected *P* values (PROC GLM; SAS Institute 2001).

Results

Self-selection

Selective intake of protein and carbohydrates was evident in both populations, but the Alaskan grasshoppers were more selective than the grasshoppers from Idaho (Fig. 1). In both populations, sex and diet choice had significant effects on

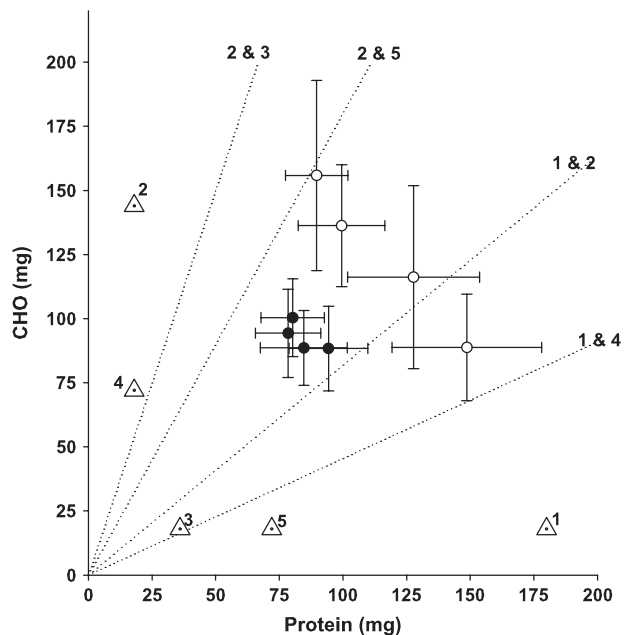


Fig. 1. Quantities of protein and carbohydrate (CHO) consumed by grasshoppers during the fourth through fifth stadia when offered the choice of two foods differing in concentrations of protein and carbohydrates. Each circle (Idaho, open circles; Alaska, filled circles) represents the group mean ($n = 18$). Error bars represent one SD. Dotted triangles indicate the amount of protein and carbohydrate in 200 mg of each of the five diets. Dotted lines represent the ratios of protein:carbohydrate if two diets offered together were eaten in equal amounts (i.e. no self-selection).

consumption of carbohydrate and protein but the relative importance of the effects were reversed in the two populations (MANOVA: Alaska, sex $F_{2,46} = 32.1$, $P < 0.0001$, diet choice $F_{6,92} = 4.8$, $P = 0.0003$; Idaho, sex $F_{2,47} = 4.9$, $P = 0.012$, diet choice, $F_{6,94} = 15.6$, $P < 0.0001$). The effects of trial and all interactions were nonsignificant ($P > 0.10$). Consumption of protein and carbohydrate was more strongly affected by the choice of diets presented to the grasshoppers in the Idaho population than in the population from Alaska (Table 1). The null hypothesis of no difference between the protein : carbohydrate ratio in the combined foods and that consumed by grasshoppers was rejected for every pair of diets presented to the grasshoppers ($t > 3.8$, $P < 0.01$), except for the Idaho grasshoppers presented with diets 1 & 2 ($t = 0.3$, $P > 0.10$). The coefficient of variation (CV) for carbohydrate consumption across all diet choices was greater for the Idaho population than the Alaskan population (17.6 and 31.3 for the Alaskan and Idaho grasshoppers, respectively, $Z = 4.4$, $P < 0.01$). Differences in variability between the two populations were not as great for protein consumption, but were still significant (CV = 18.4 and 27.4 for the Alaskan and Idaho grasshoppers, respectively, $Z = 3.2$, $P < 0.05$). The overall intake target ratios of protein : carbohydrate intake, averaged over all diet choices, was 0.90 and 0.95 for the Alaska and Idaho populations, respectively.

Intake on imbalanced diets

When protein and carbohydrate consumption by grasshoppers restricted to a series of single diets was plotted, both populations exhibited an approximately linear array of group means (Fig. 2). Regression analysis of carbohydrate consumption on protein consumption resulted in linear, negative slopes not significantly different from one (slope \pm SE -1.07 ± 0.13 , $F_{1,79} = 67.4$, $P < 0.0001$; and -0.92 ± 0.12 , $F_{1,87} = 61.1$, $P < 0.0001$, for the Alaska and Idaho populations, respectively. This pattern conforms to the 'equal distance rule' of Raubenheimer & Simpson (1999), whereby an individual's consumption of an imbalanced diet is such that differences between the intake target and actual intake are equal in the two dimensions of protein and carbohydrate.

When presented with a diet with a low, but balanced, concentration of nutrients (10:12, or 13:15), grasshoppers were able to increase consumption enough to equal the intake of macronutrients on a diet higher in nutrients (22,

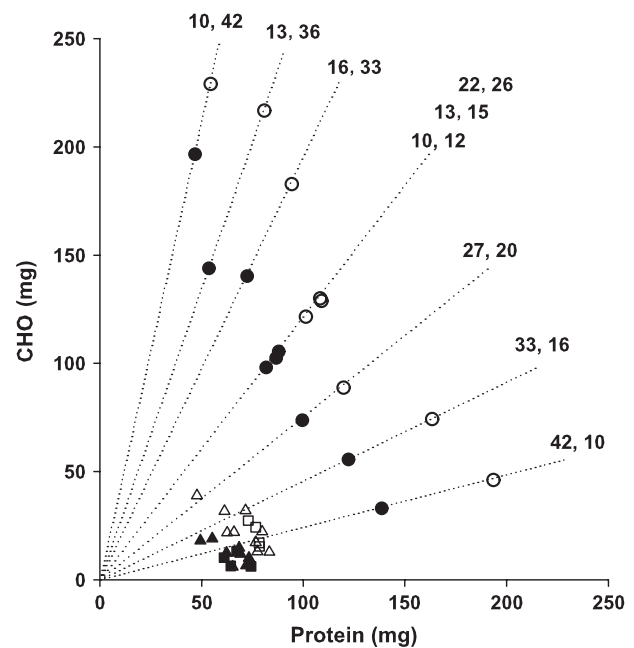


Fig. 2. Quantities of protein and carbohydrate (CHO) consumed by grasshoppers during the fourth through fifth stadia when restricted to a single diet. Each circle (Idaho, open circles; Alaska, filled circles) represents a group mean ($n = 10$). Dotted lines represent protein:carbohydrate ratios of the different diets, the concentrations (%) of which are shown at the end of the lines. Also shown are lipid content (carbohydrate-derived growth target) and nonlipid body mass (protein-derived growth target) on imbalanced diets (Idaho, open triangles; Alaska, filled triangles) and from the self-selected diets (squares).

26; Fig. 2). The amount of protein consumed on the low, but balanced, diets did not differ from that consumed on the 22:26 diet ($F_{2,49} = 0.7$, $P = 0.49$; Fig. 2). Grasshoppers consumed more of these low protein, balanced diets than the low-protein, high-carbohydrate diets, despite spending 3–7 days less in the fourth and fifth stadia. Grasshoppers consumed (mean \pm SE) 95.0 ± 2.7 mg of the 10:12 diet compared with only 50.8 ± 4.0 mg of the 10:42 diet, averaged over both populations (the diet by population interaction was not strong: $F_{1,22} = 3.5$, $P = 0.075$). When comparing the 13:36 and 13:15 diets, the population by diet interaction was stronger ($F_{1,25} = 7.0$, $P = 0.014$), but both populations consumed significantly less of the higher

Table 1. F -ratios from analysis of variance for effect of sex and diet choice on consumption of protein and carbohydrate by grasshoppers from Alaska and Idaho, U.S.A., when given a choice of foods with complementary proportions of protein and carbohydrate.

Effects	Population			Idaho d.f.		
	Alaska d.f.	Protein	Carbohydrate		Protein	Carbohydrate
Sex	1.65	34.6***	26.9***	1.67	4.5*	6.2*
Diet choice	3.65	5.5**	3.6*	3.67	26.8***	17.7***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

carbohydrate formulation than the balanced diet. Grasshoppers from Alaska consumed 68.7 ± 2.1 mg of the 13:16 diet compared with only 42.5 ± 3.8 mg of the 13:36 diet, whereas the difference was not as great in the Idaho population, 77.9 ± 2.1 and 65.5 ± 2.0 mg for diets 13:15 and 13:36, respectively.

Utilization

Despite the wide range of amounts of protein and carbohydrate consumed on the imbalanced diets, the lipid and nonlipid body composition remained relatively consistent (Fig. 2), except on the more extremely imbalanced diets with an excess of carbohydrates. On these diets, grasshopper body composition tended to have greater amounts of lipids and lower nonlipid body mass.

In the analysis of protein utilization for the choice and no-choice experiments, ANCOVA results showed population to have had a slight effect on nonlipid body mass ($F_{1,240} = 3.4$, $P = 0.066$) and both linear and quadratic terms of the covariate, the amount of protein consumed, to have influenced nonlipid body mass ($F_{1,240} = 54.6$, $P < 0.0001$ and $F_{1,240} = 28.2$, $P < 0.0001$, for the linear and quadratic terms, respectively). The interaction of both linear and quadratic terms of protein consumption with population were significant ($F_{1,240} = 5.2$, $P = 0.023$ and $F_{1,240} = 6.8$, $P = 0.010$, for the linear and quadratic terms, respectively), indicating different responses to increased protein consumption between the two populations. A plot of the data (Fig. 3B) shows that nonlipid mass increased with increasing consumption of protein but levelled off after protein consumption reached a certain amount in both populations, but nonlipid body mass of the Idaho grasshoppers reached a higher plateau.

In the analysis of carbohydrate utilization, ANCOVA results showed a strong effect of population ($F_{1,242} = 7.5$, $P = 0.0066$) and the covariate, the amount of carbohydrate consumed ($F_{1,242} = 392.8$, $P < 0.0001$) on the amount of lipids, but no significant interaction effect between population and covariate ($F_{1,242} = 2.5$, $P = 0.117$). A plot of the data (Fig. 3A) confirms that slopes were similar between the two populations, but the carcasses of the Idaho grasshoppers contained more lipid for a given amount of carbohydrate consumed than did the Alaska grasshoppers.

Performance on imbalanced diets

Survival was 90% or better on all diets except for the low protein, high carbohydrate treatments (Fig. 4A). Only five of ten grasshoppers survived to adult stage in either population on the 10:42 (%protein : %carbohydrate) diet. Six of ten Alaskan grasshoppers, and nine of ten Idaho grasshoppers survived on the 13:36 diet. Diet and population strongly affected all performance measures (Table 2). The Alaskan grasshoppers completed development in approximately 5 days less than the Idaho grasshoppers (mean \pm SE, 15.9 ± 0.35 versus 20.6 ± 0.31 days), averaged over all diets (Fig. 4B).

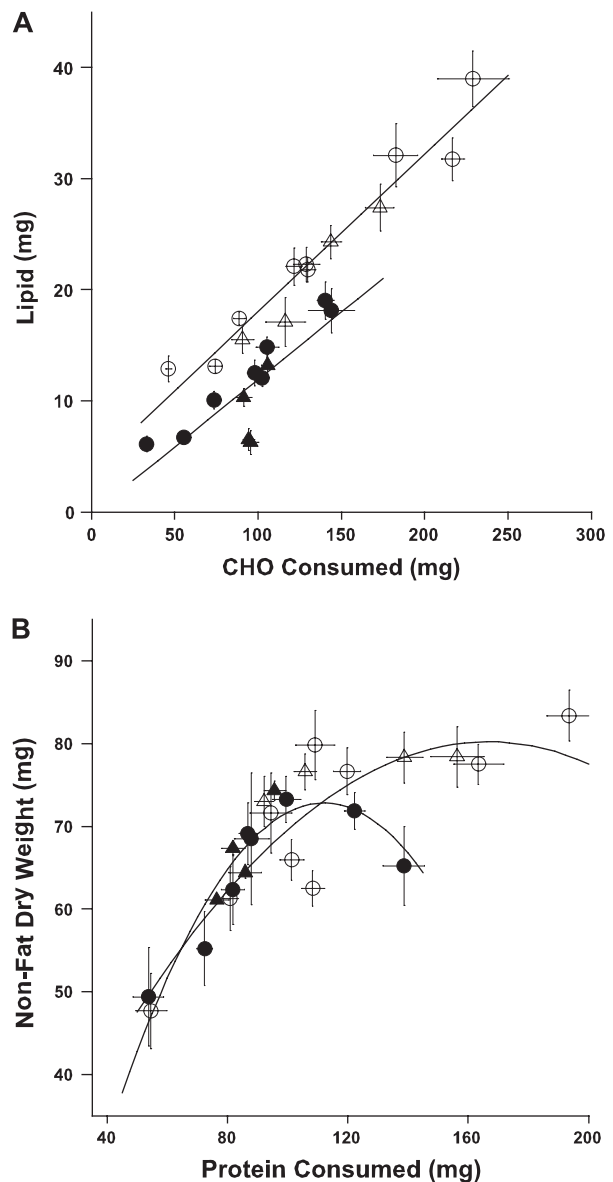


Fig. 3. Utilization plots for protein and carbohydrate (CHO) consumption. (A) Amount of lipids in newly-moulted adults versus amount of carbohydrate consumed and (B) lipid-free dry weight of newly-moulted adults versus the amount of protein consumed, as fourth and fifth instars given a choice of foods (triangles), as in Figure 1, or restricted to a single diet (circles) as in Figure 2, for two populations of *Melanoplus sanguinipes* from Alaska (filled symbols) and Idaho (open symbols). Each symbol represents Mean and SE ($n = 4-18$) of different diet treatments.

There was a significant diet by population interaction for dry mass of adults (Table 2), indicating differing responses to diet between the two populations. The total body mass of the Idaho grasshoppers was less affected by diet than was that of the Alaskan grasshoppers (Fig. 4C). The Alaskan grasshoppers had less lipid than the Idaho grasshoppers averaged over all diets, excluding the 10:42 diet for which no data for the

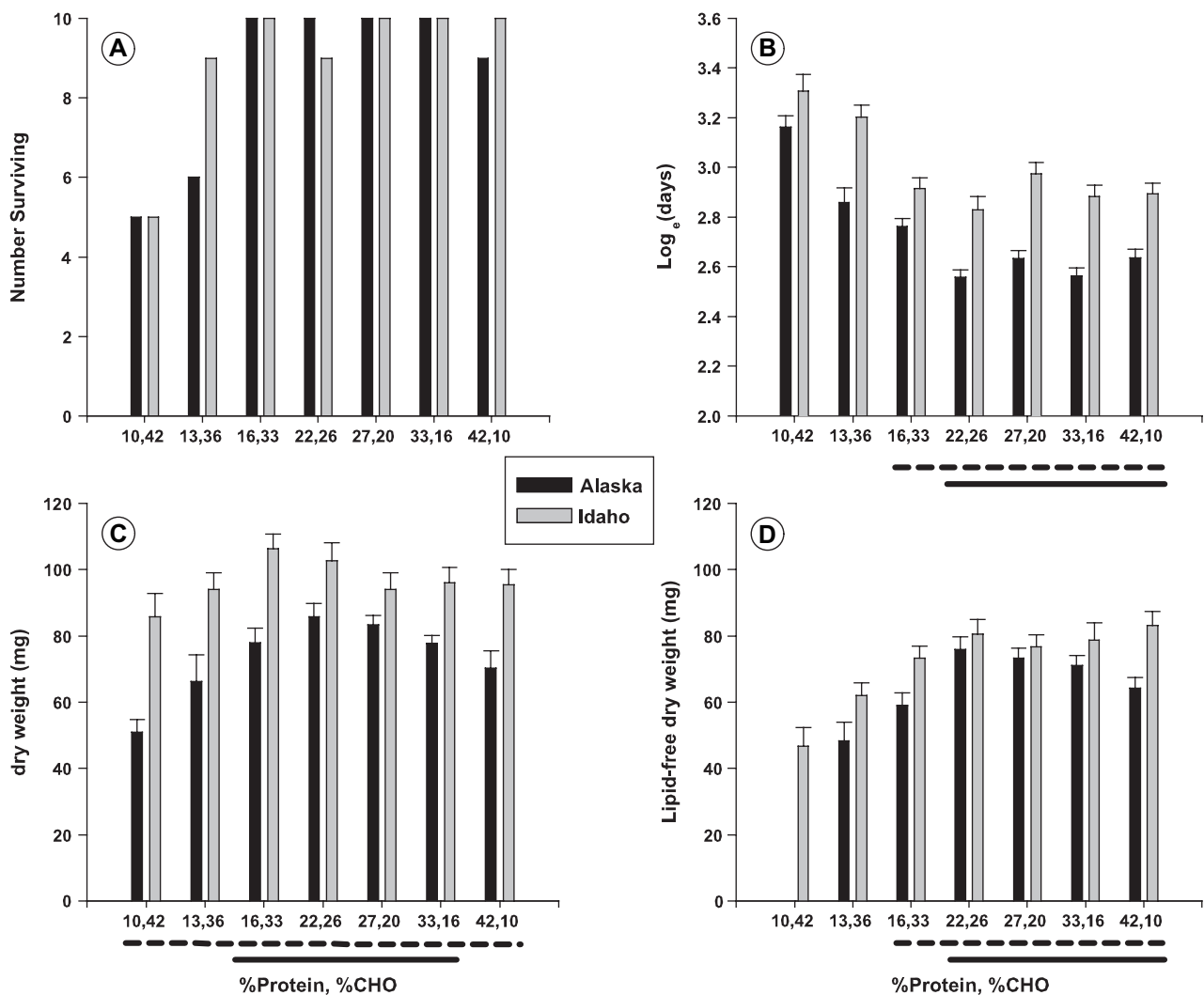


Fig. 4. Performance measures of *Melanoplus sanguinipes* from Alaska and Idaho when restricted to single diets. (A) Number (out of 10) successfully moulting to adult stage; (B) duration of fourth through fifth stadia; (C) dry carcass weight of newly-moulted adults; (D) nonlipid dry carcass weight of newly moulted adults. Bars represent least squares means and error lines indicate SE. Horizontal lines below the x-axis indicate performance plateaus [i.e. those performance measures that do not differ significantly from the shortest mean developmental time (B), or the greatest mean mass (C, D), of the seven single diets] (Idaho, dashed lines; Alaska, solid lines). CHO, carbohydrate.

Alaskan population was available (mean \pm SE, 14.8 ± 0.51 versus 19.1 ± 0.44 mg, corrected for total body mass; Fig. 3).

Lipid-free dry mass also showed a significant population by diet interaction (Table 2). The Idaho grasshoppers tended to have greater lipid-free body mass (Fig. 2D), but, on diets near the intake target ratio (diets 22 : 26 and 27 : 20), there were no significant differences between the two populations ($F_{1,18} < 2.0$, $P > 0.10$). There was no significant difference between populations on the 13 : 36 diet also, but this was likely due to the low statistical power associated with the small sample size resulting from poor survival on this diet. The population by diet by sex interaction was significant, but examination of the data did not reveal any meaningful patterns.

The performance plateau was more restricted in the Alaskan grasshoppers than in those from Idaho (Fig. 4). In the Alaskan population, the greatest total and lipid-free body mass and the shortest developmental times were achieved on the diet nearest the intake target of 0.90, but performance on diets with greater proportions of protein was similar. Performance of the Idaho grasshoppers was less well-defined and the performance plateau extended to higher proportions of carbohydrate than did the Alaskan grasshoppers (Fig. 4).

The performance of the grasshoppers on the low-protein, but balanced diets was much better than those on the low-protein, high-carbohydrate diets (Fig. 5). Survival was 90% or better on the balanced diets, developmental times were shorter and lipid-free body mass was greater than on the imbalanced diets with the same concentration of protein (Fig. 5). It thus

Table 2. *F*-ratios from analysis of variance of performance measures of *Melanoplus sanguinipes* restricted to one of a series of diets differing in protein : carbohydrate ratios.

	d.f.	Duration	Dry weight		Lipid-free
		Fourth + fifth stadia	Adults	Lipid	Dry weight
Adult dry mass		—	—	135.9***	—
Diet	6.103	27.1***	5.2***	109.0***	10.6***
Population	1.103	112.2***	71.2***	35.9***	16.6***
Sex	1.103	1.6	11.2**	2.2	9.0**
Diet × Population	6.103	1.7	2.6*	3.6**	2.6*
Diet × Sex	6.103	0.5	0.2	1.6	0.5
Population × Sex	1.103	0.3	1.8	0.3	0.7
Diet × Population × Sex	6.103	0.3	1.2	1.4	3.5**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

appears that the poor performance of grasshoppers on the diets with an excess of carbohydrates was not due solely to the low concentration of protein, but the excess carbohydrates prevented the grasshoppers from increasing consumption enough to compensate for the low levels of protein.

Discussion

Both populations of grasshoppers select diets that are within the range where performance does not suffer. Although the overall intake target of protein and carbohydrate does not differ greatly between the two populations, the self-selected intake of the Alaskan grasshoppers is more consistent than that of the Idaho grasshoppers. The protein : carbohydrate intake of the Idaho grasshoppers deviates from random expectations in three of the four choices, indicating a degree of self-selection, but their intake is contingent on the particular choices presented to them. The performance plateau of the Idaho population is correspondingly broader. It appears that whatever homeostatic mechanisms are involved in balancing of nutrient intake (Simpson & Raubenheimer, 2000), they are tuned to the width of the performance plateau. The less precise regulation of intake by the Idaho population suggests that they rely more on post-ingestive processes to increase efficiency of use of macronutrients in short supply and to cope with excesses. Such post-ingestive processes may include increased storage of excess carbohydrates as fat (Simpson *et al.*, 2002), increased respiration rates to remove excess carbohydrates (Zanotto *et al.*, 1993, 1997) and an enhanced capability to utilize amino acids as an energy source (Raubenheimer & Simpson, 2003).

It is typical for behavioural, morphological and physiological traits to complement one another. In this species, for example, darker colouration enhances the effect of behavioural thermoregulation. Nymphs from Alaska are darker and absorb a greater proportion of radiant energy than those from Idaho (Fielding & DeFoliart, 2005) and individuals from cooler environments tend to engage in behavioural thermoregulation to a greater degree than those from warmer environments (Samietz *et al.*, 2005). Although it is not possible to

generalize when working with only two populations, there appears to be a suite of traits in this species that may be correlated with food resources and physical environment of their respective habitats, including growth and developmental rates (Fielding & DeFoliart, 2007), percentage body lipids, breadth of performance plateau and flexibility of intake targets. A working hypothesis is that, due to the short growing season in Alaska, there is strong selection for rapid growth and development, which requires a fairly specific balance of nutrients. To support their more specific requirements, the Alaskan population has evolved mechanisms to optimize nutrient intake (i.e. they have become nutrient specialists). The environment from which the Idaho population was derived features a longer and much drier growing season. The grasshoppers from this region may benefit from stored lipids to prevent starvation during summer droughts. Because of the longer growing season in Idaho, rapid development may not increase fitness enough to offset the costs of increased foraging activity needed to support rapid growth and development. Costs associated with foraging activity may include increased predation risk (Beckerman *et al.*, 1997; Danner & Joern, 2003).

Although the Alaskan grasshoppers tend to be nutrient specialists compared with those from Idaho, both populations are known to be food generalists (Banfill & Brusven, 1973; Fielding & Brusven, 1992; D. J. Fielding, unpublished data), although a detailed inventory of the plant resources available in their respective habitats would be necessary for a valid comparison of the relative diet breadths between the two populations. Raubenheimer & Simpson (2003) hypothesize that generalist feeders regulate intake on single, imbalanced diets such that nutritional errors (i.e. differences in consumption from the target) are equal in the two dimensions of protein and carbohydrate. This pattern is termed the 'equal distance rule' (Raubenheimer & Simpson, 1999). Both populations in the present study conform to the equal distance rule when restricted to single, imbalanced diets. *Melanoplus sanguinipes* is polyphagous and so provides support for the hypothesis that generalist feeders may tolerate relatively large excesses of certain nutrients because their broad host range increases the likelihood of their encountering a food item with a complementary mix of nutrients.

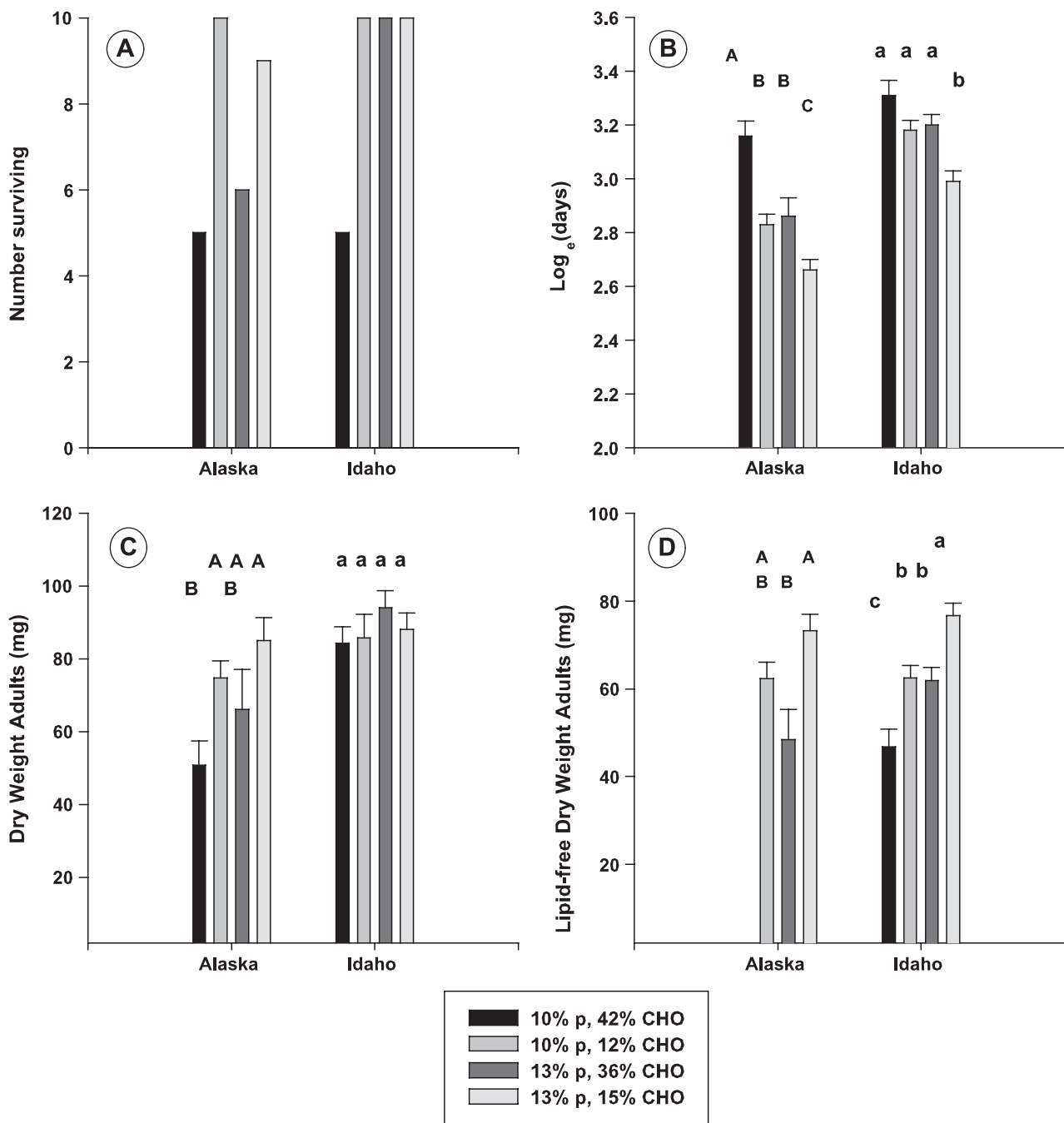


Fig. 5. Comparison of performance measures of *Melanoplus sanguinipes* from Alaska and Idaho on balanced, and imbalanced, low-protein diets. Bars represent least squares means and error lines indicate SE. Bars within a population with the same letter above them do not differ significantly (Bonferroni corrected $P < 0.05$). CHO, carbohydrate.

An understanding of the nutritional basis of pest population demographics is important for predicting outbreaks, and is also relevant to investigations of pest population responses to elevated CO_2 associated with climate change. Lower protein content of host plants associated with a rise in atmospheric CO_2 is not always detrimental to insects. For example,

Barbehenn *et al.* (2004) report the response of *M. sanguinipes* to altered host plant quality due to elevated levels of CO_2 . Protein levels in the leaves of a C_3 grass decline from 27.9% to 22.8% when CO_2 is doubled, but grasshopper consumption and growth is not affected. Along with the decline in protein, carbohydrate increases from 14.5% to 23.1%. This results in

a change in the protein : carbohydrate ratio from 1.92 to 0.99, which is closer to the target intake determined for this species in the present study (0.90–0.95). The overall concentration of macronutrients was well above levels that might be limiting. Therefore, increased CO₂ may actually improve host plant quality for these grasshoppers. The performance plateaus identified in the present study (Fig. 2) suggest that more drastic changes in leaf chemistry would need to occur before grasshopper fitness is seriously affected. It is possible that, under some conditions where leaf protein is normally low, such as is often the case on semi-arid grasslands, any further decline in leaf protein could begin to affect grasshopper demographics, especially if carbohydrates increase concomitantly. In the present study, excess carbohydrate prevents the grasshoppers from increasing consumption to compensate for low concentrations of protein. In natural foods, however, it is likely that a low concentration of nitrogen would limit photosynthesis such that carbohydrates would not accumulate to such high levels.

Subtle changes in individual performance (growth, survival, fecundity) may result in large demographic responses, especially in insect pests with high potential fertility. In a study by Joern & Behmer (1998), fecundity of *M. sanguinipes* peaks at approximately 4% N (or approximately 25% protein) and declines linearly with increasing carbohydrate, but no significant interaction is found between nitrogen and carbohydrate. The results of the present study imply that grasshopper population responses to host plant quality, as influenced by short-term weather events or by long-term climate change, may depend on the physiological attributes of the particular population in question as much as differences among grasshopper species.

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